Generic Ionizing Radiation Quarantine Treatments Against Fruit Flies (Diptera: Tephritidae) Proposed

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J. Econ. Entomol. 95(5): 893–901 (2002)

ABSTRACT Tephritid fruit flies comprise the most important group of quarantined pests of fresh produce. Most quarantine treatments of fresh agricultural commodities are directed against these pests, and considerable effort in detection, trapping, and population control is expended worldwide to prevent these pests from invading new territories. Ionizing radiation has been studied for 70 yr for its possible use as a quarantine treatment against fruit flies, but has only been applied commercially on a limited basis since 1995. The treatment has great potential and will probably be used extensively in the future as it is tolerated by more species of fruits than any other major treatment. The U.S. Department Agriculture, Animal and Plant Health Inspection Service only recently proposed allowing irradiation for fresh agricultural imports from other countries, and other countries are studying proposals to do likewise. In 1991, the International Consultative Group on Food Irradiation recommended a generic dose against all tephritid fruit flies of 150 Gy. This article examines the literature dealing with irradiation quarantine treatments against fruit flies and recommends minimum absorbed doses of 70 Gy for *Anastrepha* spp., 101 Gy for *Bactrocera jarvisi* and *B. tryoni*, and 150 Gy for all Tephritidae except when fruits have been stored in hypoxic atmospheres.

KEY WORDS fruit flies, gamma radiation, disinfestation, phytosanitary treatment, commodity treatment, postharvest

TEPHRITID FRUIT FLIES are considered the most important group of quarantined insects for their ability to render significant portions of fruit harvests unmarketable and for the extra burden placed on fruit production by detection, monitoring, and control programs that must be in place often despite the pests not actually being present to any significant degree. Large sums of money are spent to eradicate incipient populations when exotic fruit flies are discovered in new areas. For example, US $35 million was spent in Florida in 1997–1998 to eradicate outbreaks of Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann).

Postharvest quarantine treatments are used to disinfest imported hosts of fruit flies to prevent the pests from being transported across quarantine barriers (Hallman 2002). Traditional quarantine treatments against fruit flies are cold storage, fumigation with methyl bromide, and various heat treatments with insecticidal dips used in some countries. Ethylene dibromide is generally no longer used for fumigation as it has been deemed a carcinogen and mutagen, as well as causing reproductive disorders in mammals. Ionizing irradiation has been used as a quarantine treatment in the United States on a few occasions beginning in 1986. Since 1995, modest but continual amounts of fruits from Hawaii have been irradiated for quarantine purposes against four species of tephritids upon shipment to the continental United States. Since 1999 guavas, *Psidium guajava* L., have been irradiated with a minimum absorbed dose of 150 Gy against the Caribbean fruit fly, *Anastrepha suspensa* (Loew), for shipment to Texas and California. Irradiation has several advantages over other quarantine treatments. One of the biggest is that it is tolerated by more fruits than any other treatment at the doses required against fruit flies (Hallman 1999). The technique has great potential as a quarantine treatment. The U.S. Animal and Plant Health Inspection Service has recently proposed quarantine treatment doses for 11 tephritids regardless of host and for mango seed weevil, *Cryptorhynchus mangiferae* (F.), in mangoes (APHIS 2000). Other countries, especially those from the Association of Southeastern Asian Nations and other countries in Asia, the Pacific, and Latin America, have either accepted irradiation in principle as a quarantine treatment, or are seriously considering it. The World Trade Organization (WTO), through its Agreement on the Application of Sanitary and Phytosanitary Measures (SPS), does not permit member countries to reject phytosanitary measures, such as ionizing radiation, which are safe and proven to work. Currently, the Interim Commission on Phytosanitary Measures

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(ICPM, the standard setting body of the International Plant Protection Convention, or IPPC) is developing an international standard on irradiation. Irradiation has the potential to provide quarantine treatments for a number of fruit fly hosts that cannot be treated by any other means due to intolerance of the commodity to the treatment. It also is less damaging to fruits than other treatments currently used, such as hot water immersion of mangoes. The quality of some imported fruits using irradiation should improve because it can be used on fruits picked at a latter stage in the ripening process than other treatments. The use of irradiation should open up new markets for growers of tropical fruits.

Conducting efficacy treatments against all of the fruit fly/commodity combinations that may potentially be transported across quarantine barriers would place a considerable burden on the countries that produce these commodities, many of which are from the developing world. These countries do not have sufficient resources to develop efficacious quarantine treatments. Some commodities are produced in small amounts that would be insufficient or costly to sacrifice to research. Other fruits are poor hosts of fruit flies, hence, cannot be infested with sufficient numbers of flies to develop a quarantine treatment.

Dozens of fruit fly species may infest fruits that have potential to be traded across quarantine barriers; also, new species are being discovered. If generic radiation doses, i.e., single doses used across a number of applications, could be established for all fruit-infesting tephritids, or key groups of them, quarantine treatment research and application would be greatly simplified. The objective of this article is to determine if sufficient information has been generated to recommend generic irradiation quarantine treatments against tephritid fruit flies and to make these recommendations if feasible.

History. Although the application of ionizing irradiation as a quarantine treatment against tephritid fruit flies has been modest, the research record is extensive. Koidsumi (1930) seems to have been the first to study ionizing radiation as a quarantine treatment over 70 yr ago on Formosa. He found that prevention of adult emergence of oriental fruit fly, *Bactrocera dorsalis* Hendel, and melon fly, *B. cucurbitae* Coquillett, immatures could be achieved with relatively low doses of radiation and that tolerance to radiation increased as the insects developed. The field of quarantine treatments was in its infancy when he published his work with irradiation. Only 1 yr before, cold and vapor heat quarantine treatments were used for the first time to disinfest citrus during the earliest Mediterranean fruit fly outbreak in Florida. In 1949, irradiation began to be investigated as a quarantine treatment against fruit flies in Hawaii (Balock et al. 1956). By the end of the 1960s, research was underway on a number of fruit flies in various countries, and a substantial body of information on the efficacy of irradiation as a quarantine treatment was being generated (Burditt 1994).

Table 1. Measurable criteria for determining efficacy of irradiation quarantine treatments against tephritids, in decreasing order of dose required

Criterion	Dose expected
Cause acute mortality of eggs and larvae	>1 kGy
Prevent pupariation of 3rd instars	≥1 kGy
Prevent pupation of 3rd instars	~0.2 kGy
Prevent adult formation from 3rd instars	~0.15 kGy
Prevent adult emergence from 3rd instars	~0.1 kGy
Prevent adults capable of flight from 3rd instars	~0.1 kGy
Sterilize 3rd instars	≤0.1 kGy
Prevent reproduction of females from 3rd instars	<0.1 kGy

The International Consultative Group on Food Irradiation (ICGFI) is an international organization that evaluates global developments on food irradiation and advises sponsoring organizations (United Nations Food and Agriculture Organization, World Health Organization, and the International Atomic Energy Agency) and the 46 member countries (including the United States). Through an international task force convened in 1991, ICGFI recommended a generic dose of 150 Gy for all tephritid fruit flies regardless of host (ICGFI 1991). This was based on research done in several countries during the 1980s and did not take into account previous research or, of course, research conducted since 1991.

The measure of efficacy of an ionizing radiation disinfestation treatment against tephritids is normally prevention of adult emergence from eggs and larvae that are irradiated in fruits (Hallman 1999). Efficacy of quarantine treatments against fruit flies usually must be confirmed by treating large numbers of insects of the most tolerant stage present in the commodity with no survivors. In the United States, this requirement has historically been interpreted to mean that at least 93,600 fruit flies of the most radiotolerant stage should be treated with no survivors to achieve the level of control of $LD_{99.9968}$ (referred to as probit 9) at the 95% confidence interval (Couey and Chew 1986).

Objective of Irradiation Treatment. The goal of quarantine treatments is to prevent the establishment of new pests while allowing for trade in commodities that are hosts of quarantine pests. This is accomplished by almost all quarantine treatments through acute mortality of all stages of the quarantined pests present in the treated commodity. Irradiation is unique as a quarantine treatment in that little acute mortality can be expected to occur at doses tolerated by fresh commodities. But acute mortality is not necessary to prevent the establishment of new pests; prevention of further development or reproduction will suffice, and irradiation can accomplish that for fruit flies at doses tolerated by most fruits. Several objectives of irradiation quarantine treatments against tephritids can be postulated (Table 1). Of these, the first two, acute mortality and prevention of pupariation, are not practical. For example, Arthur et al. (1991) estimated that 1 kGy would be necessary to prevent 8-d-old *Anastrepha fraterculus* (Wiedemann) larvae from emerging from irradiated mangoes. Even then, the larvae may

Table 2. Comparison of estimated doses to provide $ED_{99.9968}$-level prevention of adult emergence from tephritid third instars irradiated while infesting fruits or in vitro

Species	Dose (Gy) for $ED_{99.9968}$-level control		References
	In fruit	In vitro	
Anastrepha fraterculus	≈50	16	Arthur et al. (1989, 1991), Duarte et al. (1993)
A. ludens	60	16	Bustos et al. (1992), Hallman and Worley (1999), Hallman and Martinez (2001)
A. obliqua	60	14	Bustos et al. (1992), Hallman and Worley (1999)
Bactrocera dorsalis	250	≈40	Balock et al. (1963), Seo et al. (1973)
Ceratitis capitata	150–225	≈35	Balock et al. (1963), Seo et al. (1973), Bustos et al. (1992), Mansour and Franz (1996)

Doses are results of testing many thousands of insects except in the case of *A. fraterculus*. Approximations (≈) result from small sample size (*A. fraterculus*) or the effective dose apparently lying between two tested doses.

have survived for some time before they died indicating that the dose for acute mortality would be higher. The dose to prevent pupariation of 95% of *B. dorsalis* and *B. cucurbitae* third instars irradiated while in rearing medium was estimated to be 1.6 kGy (Balock et al. 1963); that dose is probably even higher for third instars in fruits (Hallman and Worley 1999).

Criteria for Acceptable Tests

Essential information for acceptable tests of irradiation as a quarantine treatment is discussed in Hallman (2001). This information includes correct dosimetry, studies with the most tolerant stage that could be found in transported commodities, and control organisms that perform within acceptable limits. Two of these points are further clarified below. Sometimes research reports give insufficient methodology to judge if the research contains no faults that might jeopardize validity. One key clue calling into question research conducted with tephritids is adult emergence at doses to third instars that give low pupariation rates. Pupariation is a fairly simple metamorphic process, longitudinal muscular contraction of the cuticle followed by phenolic tanning. It does not require much protein-driven development on the part of the late third instar; hence, it is not easily stopped by irradiation at doses near those that would stop the next growth stage, pupation (Table 1). Therefore, studies that show adult emergence at anything but pupariation rates that are similar to those of unirradiated controls are suspect.

Irradiation in Vitro Versus in Vivo. Irradiation of fruit flies for development of quarantine treatments has been done in three ways: (1) In vitro with or without a semi-artificial rearing medium, (2) rearing on one medium followed by insertion into a fruit for irradiation, and (3) rearing and irradiation in the fruit. Because fruit flies irradiated using the first two techniques have often been reported to be more susceptible to irradiation than those reared and irradiated inside fruits (Table 2), the latter technique, being the closest to the natural situation, is more appropriate for irradiation quarantine treatment research.

Most Radiotolerant Infesting Stage. In general, insects increase in tolerance to radiation as they develop (Hallman 2000). Feeding third instars are the most radiotolerant stage of fruit fly that may be found associated with a transported fruit and are the stage that should be used when developing irradiation quarantine treatments against tephritids. Some research was done with larval stages that were probably not third instars. Balock et al. (1963) estimated that, at most, middle-aged larvae of three species of fruit flies required only a 15% higher dose to achieve the same level of prevention of adult emergence as late third instars. Therefore, studies done with late second or early third instars would be only moderately (≈15%) less tolerant than late third instars and might be used to determine quarantine treatment doses.

Doses for Quarantine Control of Tephritids

Table 3 lists all of the irradiation quarantine treatment studies done with tephritids and gives estimated doses required for near 99.9968% levels of control (effective dose or $ED_{99.9968}$). Control is defined as prevention of the adult stage capable of flight. The allowance for flies capable of flight but sterile would permit lower doses to be applied but would also risk the possibility that any flies emerging from properly treated fruits might be found in surveying traps, which could trigger an expensive regulatory response. This literature has been largely reviewed in Hallman (2000), and the reader is directed there for details. Further clarification pertinent to generic radiation doses against tephritids is provided here.

Studies done with the neotropical genus *Anastrepha* suggest that irradiation quarantine treatments of fruits infested with species of this genus may be accomplished with a dose of <100 Gy. Hallman and Martinez (2001) found that 69 Gy achieved 99.9968% security against the Mexican fruit fly, *A. ludens* (Loew), at the 95% level of confidence based on prevention of adult emergence from irradiated third instars in grapefruits. Bustos et al. (1992) found very similar results with *A. ludens* in mangoes. Although the large scale testing was done at 100 Gy, a lower dose appears likely to provide 99.9968% control. The data points that are common between the two studies (30, 40, and 60 Gy) gave somewhat similar results: 1.1, 0.11, and 0% adult emergence, respectively (Bustos et al. 1992), and 1.4,

Table 3. Estimated doses to provide $ED_{99.9968}$-level quarantine security (prevention of emergence of normal-looking adults) against tephritids irradiated while infesting fruits

Species	Fruit host	Possible dose (Gy) for $ED_{99.9968}$-level security	No. insects tested at possible dose	Reference
Anastrepha fraterculus	Apple, *Malus domestica* Borkhausen	≈25	≈70	Arthur and Wiendl (1996)
	Mango, *Mangifera indica* L.	≈50	≈100	Arthur et al. (1991)
	Uvalha, *Eugenia uvalha* Camb.	≈50	≈90	Arthur et al. (1989)
A. ludens	Grapefruit, *Citrus paradisi* Macf.	69	95,000	Hallman and Martinez (2001)
	Mango	60	4,194	Bustos et al. (1992)
A. obliqua	Carambola, *Averrhoa carambola* L.	≈50	88	Arthur and Wiendl (1994)
	Mango	60	5,513	Bustos et al. (1992)
A. serpentina	Mango	60	4,025	Bustos et al. (1992)
A. suspensa	Carambola	50	>100,000	Gould and von Windeguth (1991)
	Grapefruit	>300	4,840	von Windeguth (1982)
	Grapefruit	≈50	2,877	von Windeguth and Ismail (1987)
	Grapefruit	≫75	325	Burditt et al. (1981)
	Mango	>55	2,437	von Windeguth (1986)
Bactrocera dorsalis	Mango	150	173,000	Komson et al. (1992)
	Papaya	250	620,000	Seo et al. (1973)
B. near *dorsalis*	Mango	100	131,000	Manoto et al. (1992)
B. near *dorsalis*	Mango	100	1,500	Vijaysegaran et al. (1992)
B. jarvisi	Mango	≤101	138,600	Heather et al. (1991)
B. tryoni	Cherry, *Prunus avium* L.	≤75	1,390	Jessup (1990)
	Mango	≤101	153,800	Heather et al. (1991)
	Orange	20	80–100	Macfarlane (1966)
	Orange; avocado, *Persea americana* Mill.	75	24,700	Rigney and Wills (1985)
B. zonata	Guava	55	≈800	Haque and Ahmad (1967)
Ceratitis capitata	Mango	≤150	100,000	Bustos et al. (1992)
	Mango	≤100	81,600	Z. Torres (personal communication)
	Orange	>200 <400	?	Fésüs et al. (1981)
	Papaya	250	110,800	Seo et al. (1973)
	Papaya	≤218	70,400	Seo et al. (1973)
	Papaya; persimmon, *Diospyros khaki* L.	≈500	?	Suplicy Filho et al. (1987)
	Peach, *Prunus persica* (L.) Batsch	50	≈150	Arthur et al. (1993a, 1993b)
Rhagoletis indifferens	Cherry	18	474	Burditt and Hungate (1988)
R. pomonella	Apple	20	<2,000	Hallman and Thomas (1999)

Doses estimated through direct comparison of results, not statistical analyses.

0.0005, and 0% adult emergence, respectively (Hallman and Martinez 2001). Results of probit analyses were different; the $ED_{99.9968}$ estimate in Bustos et al. (1992) was 106 Gy (normal probability density function using log10 of dose) versus the estimate of 52 Gy of Hallman and Martinez (2001). Bustos et al. (1992) must have felt that their estimates were excessive because they did confirmatory tests using ≈100,000 insects for all three species of *Anastrepha* studied at 100 Gy, although $ED_{99.9968}$ estimates for *A. ludens, A. obliqua* (Macquart), and *A. serpentina* (Wiedemann) were 106, 133, and 136 Gy, respectively. If these estimates were accurate, there would have been a good chance of finding adult survivors for all three of these species after 100,000 insects were tested at 100 Gy, yet none were found. $ED_{99.9968}$-level security was found for *A. suspensa* after large scale testing at 50 Gy (Gould and von Windeguth 1991). In this case, few of the insects tested were in the most tolerant stage (late third instar), as they were irradiated 0–9 d after oviposition. Nonetheless, these studies plus others done with *A. fraterculus* and *A. obliqua* in Brazil (Table 3) suggest that one low quarantine treatment dose against all species of the strictly American genus *Anastrepha* for any fruit host may be feasible. This achievement would facilitate fruit exportations from the tropical and subtropical Americas in those areas where other species of fruit flies are not of concern. Of those studies reported in Table 3, those with ≤100 insects would have little confidence. The study by Burditt et al. (1981) indicating that the dose for $ED_{99.9968}$-level security is >75 Gy is contradictory because while at 75 Gy adult emergence from irradiated eggs and larvae in grapefruits was 1%, at lower doses (a total of 2,421 insects subjected to 25–50 Gy) no adults emerged. It is further suspicious because the pupariation rate at 75 Gy was only 3% while at 100 Gy it was 23%. Based on these inconsistencies it is quite plausible that the three adults found at 75 Gy were due to contamination or some other problem. The report by von Windeguth (1982) that estimates a dose to prevent adult emergence of *A. suspensa* of >300 Gy is an outlier with respect to the rest of the studies. The logistics of this study involved fruit infestation in Miami, FL, trucking to Albuquerque, NM, for treatment, and return to Miami for evaluation, with the 7-d trip repeated thrice.

One questionable result of that test was the low pupariation rate found in the irradiated grapefruits; of an estimated 9,707 insects irradiated with a target dose of 300 Gy, only 149 (1.5%) pupariated and one adult emerged.

The rest of the studies in which ≥2,400 insects were used are in quite close agreement; 50–70 Gy would provide $ED_{99.9968}$-level security against *Anastrepha* in fruits. Doses reported are likely often centerline estimates made when the irradiators were calibrated (real values would not always end in 0 or 5!). Actual maximum doses absorbed by the insects in these irradiators were probably at least 15% greater than the reported doses, given the known dose uniformity ratios in many of the irradiators used (Gammacell 220 or similar). We believe that large-scale confirmatory tests with late third instars would tend to place the minimum absorbed dose required for $ED_{99.9968}$-level security against *Anastrepha* near 70 Gy.

The earliest irradiation quarantine treatment research was conducted with fruit flies of the Asian genus *Bactrocera* (Koidsumi 1930, Balock et al. 1956). Results have been quite variable (Table 3), probably due in part to the earliness of the research, before dosimetry and other factors may have been carefully controlled.

Seo et al. (1973) is the study used to establish irradiation quarantine treatment doses of 250, 225, and 210 Gy, respectively, for *B. dorsalis*, *C. capitata*, and *B. cucurbitae* used by APHIS (APHIS 2000). The 250 Gy dose for *B. dorsalis* is substantially higher than that indicated by any other studies with any *Bactrocera* (Table 3). The dose for *B. cucurbitae* was set at 210 Gy because it was the lowest dose used by Seo et al. (1973) against that insect. These doses, as well as 225 for *C. capitata*, seem to be excessive, but examination of Seo et al. (1973) does not give a clear indication of why survivors were found for *B. dorsalis* and *C. capitata* at the reported doses of 244 and 225 Gy, respectively. It appears that careful dosimetry was conducted. The doses given are minimum doses, so some of the insects apparently received even higher doses! Care was taken to prevent hypoxia in the treated fruits, so it cannot be assumed that possible low oxygen conditions in the containers induced tolerance to radiation as sometimes occurs (Hallman 2000). Burditt (1994) speculates, but cannot verify, that contamination of irradiated fruits led to recovery of adult *B. dorsalis* and *C. capitata* from fruits irradiated with a minimum absorbed dose of up to 244 Gy by Seo et al. (1973). Reinfestation of treated commodities or contamination of treated with nontreated commodities is a concern given the large amount of fruits and insects involved and has apparently occurred (Burditt 1982, Kaneshiro et al. 1983, Burditt and Hungate 1988, Hallman 2001). In the case of *B. dorsalis*, contamination or reinfestation would have occurred in three separate experiments; a total of 22 flies emerged from ≈281,000 third instars in papayas treated with 218–244 Gy (Seo et al. 1973).

Seo et al. (1973) indicate that *B. dorsalis* is more radiotolerant than *B. cucurbitae*. In two tests at 218 and 244 Gy, no adults developed and emerged from larvae of *B. cucurbitae*, while a small percentage of adults emerged from *B. dorsalis*. Unfortunately, they do not report doses lower than 209 Gy for any of the three species studied. Lower doses with varying percentages of survivors would have helped determine if the survivors found at 218–244 Gy were expected or suspect as posttreatment contamination. What little data that are given do not appear to progress normally. At 209 Gy, there were no adult *B. dorsalis* produced from an estimated 29,265 larvae treated, while at 218 Gy there were two adults of 73,618, at 225 Gy two adults of 76,850, and at 244 Gy 17 adults of 130,156. Within this dose range it appears as if dose was directly proportional to survival! These results are consistent with a low level of reinfestation or other source of erroneous adults independent of radiation dose. It might also be expected that as the size of the experiment increases the chance for error increases, hence, no adults when 29,265 larvae were treated versus 17 adults when 130,156 were treated. But, at doses of 246–291 Gy no adults were found even though estimated total number of larvae treated in that range was 670,600, offering support that contamination was not a consistent problem. However, contamination is further suspected because most of the adults found (22 of 24) were *B. dorsalis*, the species most commonly found infesting fruit when and where this research was conducted. No adults of *B. cucurbitae* were found, and it is the species least likely to be found infesting fruit in Hawaii in the early 1970s.

The separation of the *B. dorsalis* complex into 41 species by Drew and Hancock (1994) cast doubt on the identity of the species of *Bactrocera* studied in three southeast Asian countries, as voucher specimens were not archived. However, because the fruit flies used in the research described in Thailand (Komson et al. 1992) were collected in the Bangkok area, they are probably true *B. dorsalis*, as other species listed in Thailand do not occur around Bangkok but further south in the Malay Peninsula. The authors obtained one adult *B. dorsalis* of an estimated total of >173,000 5-d-old larvae (at 27°C) infesting mangoes that were irradiated with 150 Gy. Two adults emerged from 1-d-old larvae irradiated at 75 Gy in mangosteens (the lowest dose used); no adults emerged from 3- to 7-d-old larvae so treated. The results of these studies indicate that slightly <150 Gy would be the minimum dose required to achieve the $ED_{99.9968}$ at the 95% confidence level. One survivor of 137,200 treated would be the minimum possible number to achieve the $ED_{99.9968}$ at the 95% confidence level (Couey and Chew 1986).

Research done in the Philippines (Manoto et al. 1992) and Malaysia (Vijaysegaran et al. 1992) was probably done with *B. papayae, carambolae,* and/or *philippinensis* under the taxonomic scheme of Drew and Hancock (1994). A dose of 50 Gy applied to larvae 7 d postinfestation in mangoes in the Philippines resulted in 2.25% adult emergence, whereas 100 Gy completely prevented adult emergence from an estimated 131,000 larvae 5–6 d postinfestation (Manoto et al. 1992). The larvae treated at 100 Gy were not the most developed possible, hence, might be expected to be more susceptible to irradiation than the larvae used in the tests with 50 Gy (7 d postinfestation). However, in detailed studies done with *C. capitata*, *B. dorsalis*, and *B. cucurbitae* in Hawaii, there was essentially no difference in susceptibility of oldest larvae and those 1 d younger (Balock et al. 1963). Therefore, it would be reasonable to expect that 100 Gy was somewhat more than necessary to provide the $ED_{99.9968}$ at the 95% confidence level for the species of *Bactrocera* irradiated in mangoes in the Philippines. A dose of 80 Gy applied to carambolas infested with early third-instar *Bactrocera* sp. in Malaysia resulted in the emergence of one adult from a total of ≈1,500 larvae (Vijaysegaran et al. 1992).

Many studies have been done with *C. capitata* with quite variable results. Besides those reviewed previously by Hallman (2000), Kaneshiro et al. (1983) obtained two adults from ≈26,000 third-instar *C. capitata* irradiated with 500 or 600 Gy in stone fruits. Both adults were fertile, and the authors suspected that they resulted from accidental reinfestation after irradiation.

Five of 106,300 third instars in mangoes irradiated with 150 Gy survived to the adult stage (Bustos et al. 1992). However, the five survivors all occurred within the first 5,300 larvae tested; no survivors occurred in the rest of the 101,000 larvae irradiated. Data from dose–response testing showed that the level of adult emergence remained stable at 0.09–0.16% from 80–150 Gy instead of gradually declining as the dose increased. This type of relationship between dose and response is indicative of a mixed population where some of the individuals are significantly more tolerant of irradiation than the rest. But what also may have happened is that there was a low level of reinfestation of fruits in the large *C. capitata* rearing facility in Chiapas, Mexico, where the research was carried out (M. E. Bustos, personal communication). The irradiated mangoes were not completely protected from escaped flies in the facility until after the dose–response testing was initiated (the first 5,300 larvae tested), possibly resulting in some adult emergence due to reinfestation. The fact that no adults emerged during the large-scale testing of 101,000 third instars, when the mangoes were adequately protected from reinfestation, should be sufficient support of 150 Gy as a treatment that will provide quarantine security against the Mediterranean fruit fly.

New studies with Mediterranean fruit fly conducted in Peru have tested 81,600 third instars in mangoes at 100 Gy with no adult emergence (Table 3; Z. Torres, personal communication). Four studies that compared susceptibility of the Mediterranean fruit fly with that of *Anastrepha* spp. found that the former was more radiotolerant than the latter at a ratio of ≈1.4:1 (Faria 1989, Raga 1990, Bustos et al. 1992, Duarte et al. 1993). Therefore, if 70 Gy seems sufficient to prevent adult emergence of *Anastrepha* spp., then ≈100 Gy might be sufficient for Mediterranean fruit fly.

Farrar (1999) argues that many published reports on radiation quarantine treatment research are of little value because of insufficient information about the dosimetry and uncertainties in measuring dose. In some published papers, the dose reported is often the minimum dose to which insects were exposed. This is because researchers will use the centerline dose as the true dose, ignoring the higher dose received by insects closer to the gamma source rods. Dose-attenuation by the fruit may not be measured. This experience with the Mediterranean fruit fly clearly demonstrates the need for careful dose measurement and experimental methodology in general.

Fruit Host and Treatment Efficacy. Host does not seem to affect susceptibility of fruit fly larvae to irradiation (Table 3). With two dubious exceptions concerning *A. suspensa* in grapefruit that have been discussed above, prevention of adult emergence of several species of *Anastrepha* from third instars is accomplished with 50–70 Gy regardless of fruit host. Studies with *B. tryoni* in cherry, mango, orange, and avocado require doses of 75–100 Gy to prevent adult emergence from third instars. Doses for *C. capitata* were quite variable, as discussed above, but are not likely due to host.

Recommended Generic Doses. A considerable amount of research on irradiation quarantine treatments for many species of Tephritidae has been done, and recommendations for generic treatments could be offered. The largely consistent results from five species of *Anastrepha* indicate that a generic dose of 70 Gy would suffice against that important neotropical genus of Tephritidae. A generic dose of 150 Gy for *Bactrocera* is probably warranted given that only one study (Seo et al. 1973) of a total of nine done with six different species indicates that ≤150 Gy is inadequate and should be considerably higher (250 Gy). *C. capitata* does seem to possess ≈1.4 times the radiotolerance as *Anastrepha* spp., indicating that the minimum dose to prevent adult emergence from third-instar *C. capitata* would be ≈1.4 × 70 Gy = 98 Gy. However, the results with that species are quite variable, making general conclusions a little more precarious than with *Bactrocera.* However, it is interesting to note that all of the studies published before 1988 yield quarantine doses of ≥200 Gy, whereas all of the studies published after 1988 give doses of ≤150 Gy. It is as if a factor defining all previous research changed in 1988. This factor might be the international training and support that the International Atomic Energy Agency offered to irradiation quarantine treatment researchers with research co-ordination projects beginning in the mid-1980s. We recommend a dose of 150 Gy for this important insect with the realization that the lowest possible dose seems closer to 100 Gy (Table 3; Z. Torres, personal communication).

Given that a dose of ≤150 Gy seems sufficient to provide quarantine control for all 14 species of tephritid fruit flies from four genera of economic importance listed in Table 3, we believe that there is sufficient evidence to recommend an ionizing radiation quarantine treatment minimum absorbed dose of

Table 4. Radiation doses used in tephritid sterile insect technique programs around the world (IAEA 2001)

Species	Range of minimum absorbed doses (Gy)
Anastrepha ludens	70–80
A. obliqua	80
A. suspensa	70
Bactrocera cucurbitae	50–120
B. dorsalis	90–120
B. philippinensis	50
B. tryoni	70–180[a]
Ceratitis capitata	90–145

[a] High value used when pharate adults irradiated in nitrogen atmosphere; anoxia increases radiotolerance.

150 Gy. Because fruit flies have been shown to be more radiotolerant under hypoxic conditions compared with air (Hallman 2000) it is recommended that irradiation quarantine treatments not be applied to fruits stored in hypoxic atmospheres immediately before treatment, even if the storage has been for only a short while, until research resolves any possible problems with reduced efficacy.

A further argument to support a generic dose of 150 Gy for all Tephritidae is that fruit flies used in sterile insect release (SIT) programs use lower doses, except for one occasion where *B. tryoni* in a nitrogen atmosphere was irradiated with 180 Gy (Table 4). It is well documented that radiotolerance (when measuring the same objective) is directly related to development. In a thorough review of Arthropoda, Hallman (2000) only found one apparent exception; human body louse, *Pediculus humanus humanus* L., nymphs were reportedly more tolerant of radio-induced reproductive sterility than adults (Cole et al. 1959). Furthermore, female sterility in insects, and especially Tephritidae, is often accomplished with lower doses than those required for males (Hallman 2000). SIT doses are developed to sterilize males, while doses for quarantine purposes would suffice if only the female does not reproduce, as that would prevent the establishment of the pest. The objectives in quarantine and SIT are different: prevent emergence of adults capable of flight in the former and prevent reproduction in the latter. Prevention of adult emergence from irradiated third instars is achieved with slightly lower doses than those required to prevent reproduction (Table 1). However, in SIT programs, late pharate adults and not third instars are irradiated; it would require a higher dose to prevent reproduction from pharate adults than third instars (Tables 4 and 5, respectively). The only reason prevention of adult emergence has been chosen as the measure of efficacy of radiation quarantine treatments is to prevent finding adult fruit flies in trap-monitoring programs. Prevention of reproduction would achieve the goal of stopping an imported insect from becoming established. The fact that SIT doses for all fruit flies (except one instance of 180 by for *B. tryoni* in a nitrogen atmosphere) are <150 Gy gives confidence that 150 Gy would suffice as a quarantine treatment against Tephritidae. Furthermore, quarantine security against *B. tryoni* is achieved with 75–100 Gy (Table 3). Fruit flies used in SIT programs throughout the world are released in the millions weekly over large agricultural and urban areas containing abundant hosts and favorable conditions for establishment. Much confidence is placed in their being reproductively sterile, and this is tested constantly as a matter of quality control.

Table 5. Doses to prevent reproduction of third-instar Tephritidae irradiated in fruit

Species	Dose (Gy)	Reference
Anastrepha ludens	10, 40	Benschoter and Telich 1964, Bustos et al. 1992
A. obliqua	Between 40–60	Bustos et al. 1992
A. serpentina	10	Bustos et al. 1992
Ceratitis capitata	60	Bustos et al. 1992

Recommendations for Research with Tephritidae

A series of points necessary for supporting acceptable irradiation quarantine research with Tephritidae is suggested to guide future studies. Their inclusion in much of the literature cited in this analysis no doubt would have aided in arriving at generic doses.

Voucher specimens should be filed to address future taxonomic reorganizations. Studies should be done in fruit using third instars reared in that fruit. Ensure that the late third instar is achieved before irradiation; tephritids often require more time to mature in fruit than in diet. Dosimetry should be done routinely in those parts of the load where the extremes are expected and comparable to accepted standards. Irradiations should be done to minimize the dose uniformity ratio, as the maximum absorbed dose found in the research confirming a target dose to be used in commercial application should be the minimum dose recommended for treatment. Adult emergence in the control should be ≥80%. There must be complete separation of all treatments, especially the control from the irradiated treatments, and all treatments must be protected from reinfestation by feral or laboratory flies.

Acknowledgments

James E. Throne (*Journal of Economic Entomology* Editor), is acknowledged for encouraging this study. Alan K. Dowdy (USDA, APHIS, CPHST, Raleigh, NC), Peter Follett (USDA-ARS, Hilo, HI), and Thomas Phillips (Oklahoma State University, Stillwater) are thanked for reviewing the manuscript.

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Received for publication 18 May 2001; accepted 12 May 2002.